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MORPHOLOGICAL VARIATION IN VICTORIAN, LOWLAND POPULATIONS OF *EUCALYPTUS PAUCIFLORA* SIEB. EX SPRENG.

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Abstract: The pattern of morphological variation in isolated, lowland populations of *Eucalyptus pauciflora* was analysed phenetically using multivariate classification and ordination techniques. The main aim was to discover how variable were the lowland populations in the context: (1) of the physiological and morphological variation in the species at higher altitudes; and, (2) of the scattered nature of these sites, which are possibly relics from a wider distribution during the last glaciation. Outgroups were used as aids to interpreting the patterns.

Adult morphology showed little variation at lowland sites, although some small differences were observed, and some populations showed signs of recent hybridisation with *E. obliqua* and *E. radiata*. Within the total range of *E. pauciflora* however, seedlings showed two forms with the lower altitude populations possibly showing the ancestral condition. An equivocal large population at Wartook Reservoir, Grampians, suggested that introgression between *E. pauciflora* and *E. willisii* has occurred in the past, resulting in a stable intermediate form.

Eucalyptus pauciflora Sieb. ex Spreng. (an ash species: informal subgenus *Monocalyptus*, series *Obliquae*, Pryor and Johnson 1971) is the dominant tree at high altitudes in south-eastern Australia, occurring throughout the mountains and sub-alpine areas of eastern Victoria and New South Wales, extending marginally into Queensland and occurring throughout most of central and eastern Tasmania. The altitudinal range on the mainland is from sea level to 2000 m, but largely 730-2000 m, and in Tasmania between just above sea-level to 730 m, with limited extension up to 1,276 m (Hall, Johnston & Chippendale 1970).

Lowland populations (0-700 m) principally occur in a disjunct distribution in southern Victoria (Fig. 1). Scattered amongst these lowland populations are a few higher altitude populations at approximately 1000 m (Major Mitchell Plateau, Mt. Cole, Camels Hump). Remnant trees which occur south of Mt. Gambier in South Australia, are the most westerly occurrence of the species (Boomsma & Lewis 1981). At higher altitudes, *E. pauciflora* shows considerabe morphological variation—Mueller (1879-1884) noted differences in leaf shape and fruit size in the "alpine variety"; the species later being shown to vary clinally, both morphologically and physiologically, with altitude (Pryor 1957, Green 1969, Burdon & Chilvers 1974, Slatyer 1977a, b, c, 1978, Slatyer *et al.* 1977a, b, c). This variable nature of *E. pauciflora* at higher altitudes led to the question of how variable were the isolated, lowland populations. The disjunct nature of these lowland populations has been commented on (Costermans 1981), but otherwise left unstudied. Specimens of *E. radiata* and *E. willisii* (peppermints) and *E. obliqua* (ash) were also included in this study as these taxa were suspected of being involved in hybridisation with *E. pauciflora* at some locations.

METHODS

Populations Sampled

In total, 20 populations of *E. pauciflora* were sampled (Fig. 1), ranging from the most westerly occurrence through to Wilsons Promontory. Within this range, samples of populations from isolated mountain tops, such as Mt. Cole, were also collected. Samples from Mt. Buffalo and Eildon were included as representatives of populations growing in the south-eastern alps and Tasmania respectively. Specific site localities are tabulated in Appendix 1.

In general, five trees were sampled from each locality, although the small size of some populations meant that this was not always possible. Additional samples were often taken where individuals appeared to be hybrid or morphologically variable. Trees were sampled throughout the spatial range of each population to avoid collecting closely related individuals. Fruits, buds and leaves were collected, where present, from each individual. Herbarium specimens are lodged at the University of Melbourne. Buds, and a leaf for oil gland density, were preserved in 70% alcohol; and fruits were air-dried for seed extraction and subsequent seedling growth.

Seedling Trial

Seed from 18 populations (93 parent trees) was available for the growth of seedlings. Seed was either sown directly into pots or was placed on moist filter paper in petri dishes (e.g. higher altitude populations) and stratified for 4 weeks at 4.5°C to improve germination (Hargreaves 1977). Germination in the latter occurred at a minimum one week after cold treatment. To minimise fungal growth, a small amount of Karathene was added to all petri dishes. Depending on germination rate, 1-6 seedlings were pricked into individual pots containing a general potting mixture. Each parent tree was represented by four such pots. Some seed from Powlett River (4 trees), South Australia (2 trees), Major Mitchell Plateau (1 tree) and Green Ck. Rd. (1 tree) did not germinate and appeared to be inviable. Some seed lots consisted principally of chaff and others had few viable embryos (tested by seed squash). Seedlings were grown in a heated glasshouse for 4-5 months. Pots were even-

Fig. 1—Generalised distribution of *Eucalyptus pauciflora* (stippling) in Victoria, South Australia and Tasmania, with collection sites superimposed. Abbreviations: MMP = Major Mitchell Plateau, WR = Wartook Reservoir, GCR = Green Creek Road.

tually thinned to one seedling per pot, unless segregation was apparent. During the winter, four 500 W self-balasting mercury vapour lamps, photoperiod 10/14 hr, were used to supplement daylight, and seedlings were rotated to ensure maximum effectiveness.

Seedlings of *E. obliqua* (seed from Brisbane Ranges), *E. radiata* (from Eltham) and *E. willisii* (from Grampians) were grown for comparison with seedlings from alleged hybrid populations, to check for character segregation.

Characters

Adult

To cover the inherent variability within individuals, five mature leaves and ten fruits and buds were measured per tree. Twenty-six adult characters were scored (Table 1, Fig. 2). Mean oil gland density was calculated by measuring five 8 mm grids mid-lamina of each leaf. Buds were cut longitudinally to measure pedicel length since it was difficult to measure the true length externally. The form factors, measured on a digitiser, gave an indication of leaf shape, a value of 1.0 indicating a circle and 0.0 a line.

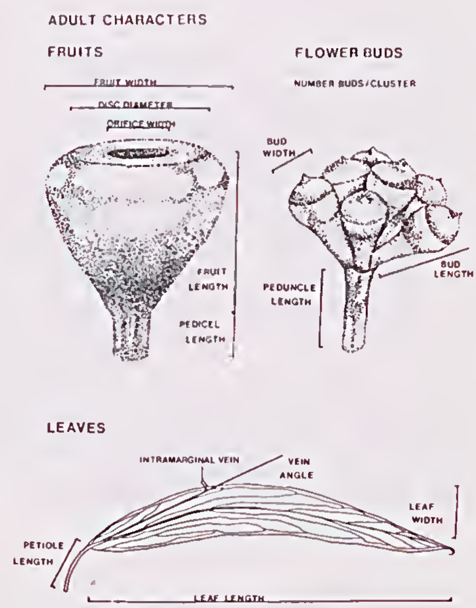

Fig. 2—Adult characters measured.

TABLE 1
ADULT CHARACTERS SCORED FOR DATA MATRICES

		State
Binary		
1. Leaves	dull/glossy	0/1
2. Leaf apex	with/without hook	0/1
3. Branchlets	non glaucous/glaucous	0/1
Multistates		
4. Fruit disc	Sunken/level/raised	1/2/3
Numerics		
5. Leaf length (mm)		
6. Leaf width (mm)		
7. Form factor		
8. Leaf thickness, mid lamina (mm)		
9. Secondary vein angle (°)		
10. Intramarginal vein (mm)		
11. Oil gland density, mid-lamina (per cm^2)		
12. Displacement (mm)		
13. Petiole length (mm)		
14. Maximum fruit width (W) (mm)		
15. Disc diameter (mm)		
16. Fruit length (L) (excluding pedicel) (mm)		
17. L/W ratio		
18. Diameter of orifice (O) (mm)		
19. O/W ratio		
20. Distance from widest part of fruit to disc edge		
21. Pedicel length (mm)		
22. Number of flower buds/cluster		
23. Bud length (including pedicel) (mm)		
24. Pedicel length (mm) buds cut in L.S		
25. Peduncle length, buds (mm)		
26. Maximum bud width (mm)		

Form Factor $= \frac{4\pi p^2}{A}$ where p = leaf perimeter, A = area

Seedlings

In total, 370 seedlings were measured for 27 characters (Table 2). The following seedling leaf stages were defined for *E. pauciflora*, counting cotyledons as node 1 (Fig. 3a). (1) Seedling leaves are opposite, in most cases petiolate, occur at nodes two to three, and only occur on seedlings. (2) Juvenile leaves are broader, opposite, petiolate, and horizontally orientated, occurring for the next one to seven nodes. They are also found as coppice. (3) Intermediate leaves show internode formation and often a 90° twist in the petiole to orientate the leaf in the vertical plane. Leaves are broad relative to adult foliage and do not show parallel veination. Other ashes, such as *E. obliqua*, incorporated in this study, show a similar sequence of seedling stages. In comparison, the peppermints have opposite, sessile juvenile leaves for an indefinite number of nodes (Fig. 3b). The distinct differences between the two types of seedlings proved useful in the identification of hybrids.

NUMERICAL ANALYSIS

Adult trees and seedling characters were analysed using phenetic, multivariate classification and ordination techniques available in the C.S.I.R.O. TAXON package (Ross 1982). Initially, a data matrix of all individuals (either adults or seedlings), was used to calculate a dissimilarity matrix between all pairs of individuals. Squared, standardised, Euclidean distance, was the dissimilarity measure generated by the program MSED. Both adults and seedlings were classified using an agglomerative system (SAHN) which minimised the incremental sum of squares. A dendrogram provided a two-dimensional representation of the hierarchy, and the program GCOM indicated which attributes were important in the classification. The program CRAMER also ranked attributes on their ability to discriminate between groups.

Ordination consists of displaying, in an efficiently reduced Euclidean space, the interrelationships of the entities under study (Williams, Dale & Lance 1971) and can reveal relationships which otherwise might be overlooked when a large amount of data is to be interpreted. An advantage of ordination is that entities can be shown in three-dimensional space, separating groups which may be clustered into two dimensions. In this case Principal Coordinates Analysis (PCOA) was employed. All adult individuals were ordinated but, due to the large numbers involved (370), seedlings were only ordinated

TABLE 2
SEEDLING CHARACTERS SCORED FOR DATA MATRICES

		State
Binary		
1. Stem wax	present/absent	0/1
2. Raised oil glands on stem	zero, few/many	0/1
3. Underleaf colour, node 2	purple/not	0/1
4. Leaf base, node 4	cordate/not cordate	0/1
5. Leaf base, node 5	cordate/non cordate	0/1
6. Leaf surface, node 4	dull/shiny	0/1
7. Leaf colour, node 4	green/blue-green	0/1
8. Leaf underside, node 5	discolourous/concolourous	0/1
9. Raised oil glands on leaf, node 4	absent/present	0/1
10. Lignotuber	absent/present	0/1
11. Leaf surface, node 6	green/blue-green	0/1
Multistates		
12. Node at which leaves alternate	nodes 3-7	1
	nodes 8-9	2
	nodes 10 and above	3
13. Node at which leaves twist	nodes 3-7	1
	nodes 8-9	2
	nodes 10 and above	3
14. Node at which leaves become petiolate	nodes 3-7	1
	nodes 8-9	2
	nodes 10 and above	3
Numerics		
15. Petiole length, node 2 (mm)		
16. Petiole length, node 4 (mm)		
17. Leaf length, node 4 (mm)		
18. Leaf width at widest part, node 4 (mm)		
19. Form factor, node 4 (mm)		
20. Oil gland density, node 4 (per cm²)		
21. Secondary vein angle, node 4 (°)		
22. Intramarginal vein (mm)		
23. Petiole length, node 5 (mm)		
24. Leaf length, node 5 (mm)		
25. Leaf width at widest part, node 5 (mm)		
26. Form factor, node 5 (mm)		
27. Petiole length, node 7 (mm)		

as group centroids (average individual for each group). Gillison's (1978) technique of displaying group centroids on the third axis as a series of graded spheres was employed. BACRIV was also used to calculate the correlation of each attribute to the axes of PCOA. A minimal spanning tree (MST), used in conjunction with the ordination of group centroids, provided a network of shortest total length connecting all points under consideration such that each point is connected with every other point by a chain of links with no closed loops occurring (Gillison 1978).

RESULTS

ADULT TREES

The classification based on all adult individuals (Fig. 4) was truncated at both the ten group and five group level. The first major dichotomy separated the peppermints, *E. radiata* and *E. willisii* (Group 5), from the ashes, *E. pauciflora* and *E. obliqua* (Groups 1-4). Characters contributing to this division were vein angle, leaf thickness and fruit size. According to GCOM, the peppermints were distinguished by a wider vein angle (20°), thinner leaves (0.22 mm) and smaller fruits (5.4 mm wide). This is in comparison with the ashes which had a vein angle of 9° (dominated by individuals of *E. pauciflora*), leaves 0.37 mm thick and fruits 8.5 mm wide on average. Of the five major groups (Fig. 4), *E. obliqua* was exclusively classified into group 4 and was characterized by a mean vein angle of 21°, oblique leaf bases and an intramarginal vein 3 mm from the leaf edge. Within *E. pauciflora*, individuals were classified into groups 1, 2 and 3: group 1 was distinguished by large fruits (9 mm wide, 9 mm long) with level discs, group 2 by uncharacteristically small fruits (6.5 mm wide, 6.5 mm long), group 3 again by large fruits (9 mm long) but with sunken discs. The division on disc position is largely artificial since the character is variable within populations, and thus some of them were split between groups 1 and 3. Cramer values for characters that best distinguish these 5 groups are shown in Table 3.

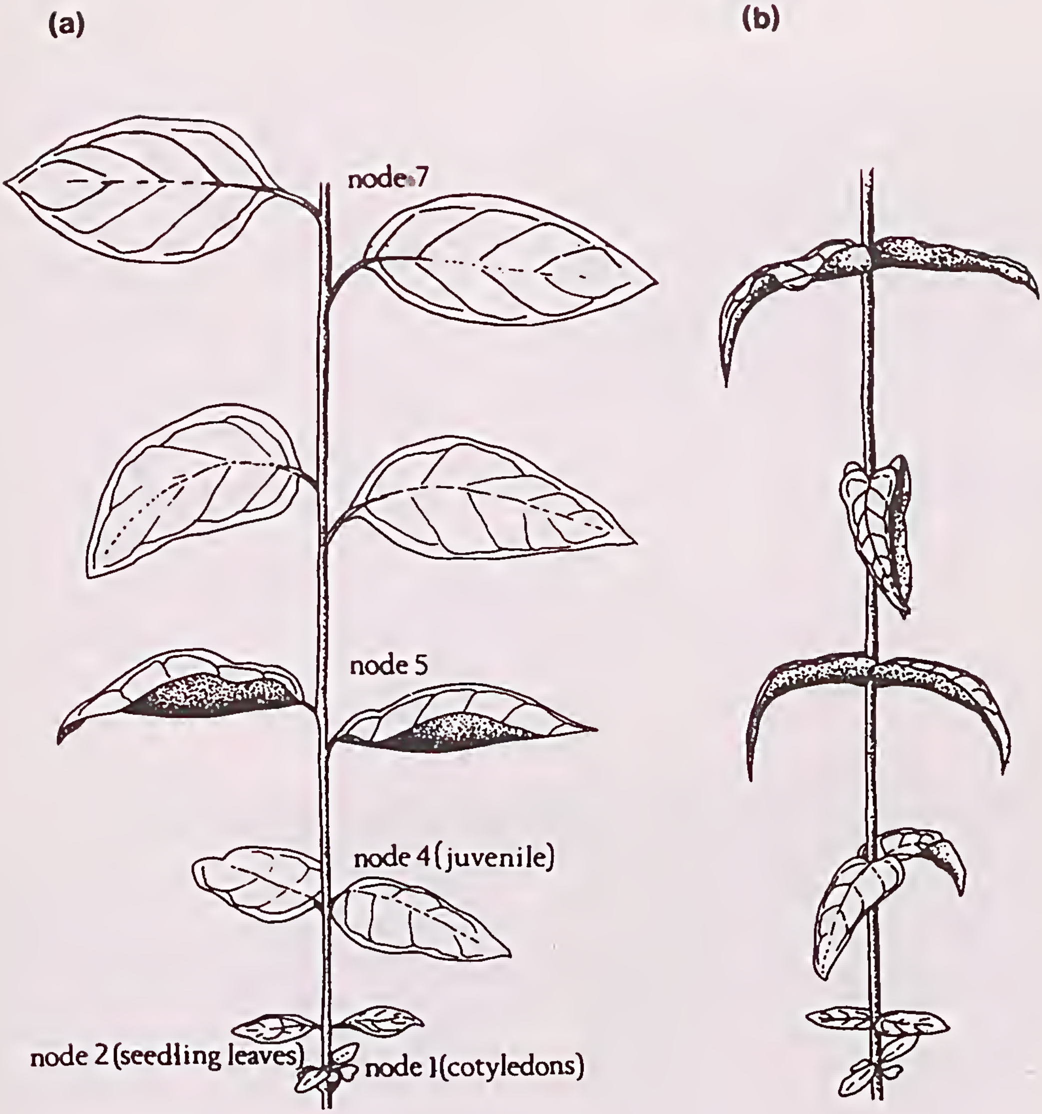

Fig. 3—(a), A typical higher altitude seedling of *E. pauciflora* showing the principal nodes measured (on all seedlings), early intranode formation and orientation in the vertical plane. (b), A typical peppermint seedling (*E. radiata*) showing sessile, opposite, juvenile leaves which occur for an indefinite number of nodes.

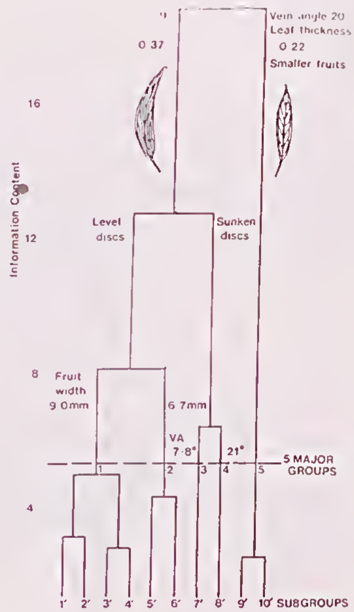

Fig. 4—Classification of 122 adults truncated at the five and ten group level; VA = vein angle. Percentage composition, on a population basis, of the five major groups is as follows. 1: Moorooduc 100%, Mt. Martha 80%, Durdidwarrah 100%, Trentham 60%, Woodend 100%, Camels Hump 100%, Digby 2 100%, Mt. Cole 50%, Yan Yean 100%, Green Ck. Rd. (Grampians) 40%, Mt. Buffalo 60%, Wilsons Promontory 80%, Cape Schanck 40%, Powlett River 40%. 2: Digby 1 100%, Major Mitchell Plateau 100%, Wartook Reservoir (Grampians) 100%, South Australia 50%. 3: Tasmania 80%, Powlett River 40%, Drik Drik 100%, Green Ck. Rd. 40%, Mt. Cole 50%. 4: *Eucalyptus obliqua* 100%. 5: *Eucalyptus radiata* 100%, *Eucalyptus willisii* 100%.

TABLE 3

CRAMER VALUES FOR CHARACTERS THAT BEST DISTINGUISH THE FIVE MAJOR GROUPS DEFINED IN THE CLASSIFICATION OF ADULT TREES. THE MEAN VALUE IS GIVEN FOR QUANTITATIVE VALUES.

Groups		Characters	
		Maximum fruit width (mm)	Secondary vein angle (°)
1	*E. pauciflora*	9.06	8.17
2		6.68	8.52
3		9.22	7.84
4	*E. obliqua*	7.97	20.83
5	*E. radiata* *E. willisii*	5.40	20.10
		Cramer Value = 0.8635	Cramer value = 0.8423
		Disc diameter (mm)	Fruit length (mm)
1		7.54	8.59
2		5.72	6.42
3		7.01	9.52
4		4.98	9.15
5		4.38	4.77
		Cramer value = 0.8390	Cramer value = 0.8254
		Orifice diameter (mm)	Leaf thickness (mm)
1		4.30	0.39
2		3.25	0.35
3		4.26	0.35
4		3.42	0.32
5		2.41	0.22
		Cramer value = 0.7811	Cramer value = 0.7035

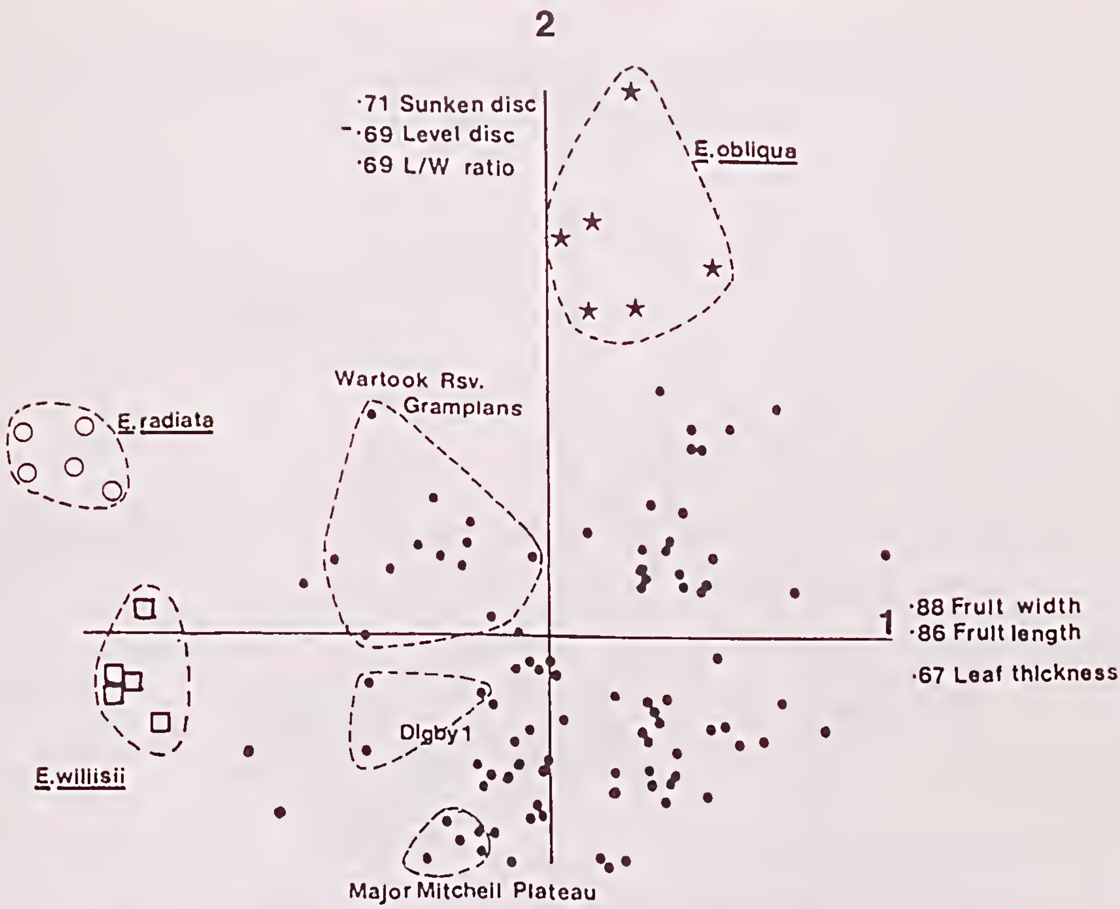

Fig. 5—Ordination of all adult individuals. Solid circles represent individuals collected as *E. pauciflora* (or putative hybrids). Character correlation coefficients are shown on axes; L/W = length/width ratio.

A comparison of the ten subgroups gives more information on the pattern of variation within *E. pauciflora*. Individual trees from any one geographic district were generally classified in the same subgroup. Five subgroups contained most of the *E. pauciflora*, and they consisted principally of the following individuals (percentage occurrence indicated): 1′, Moorooduc (100%), Mt. Martha (80%) and Durdidwarrah (100%); 2′, Woodend (100%), Trentham (60%), Camels Hump (100%), Digby 2 (100%) and Mt. Cole (50%); 3′, Wilson's Promontory (80%) and Yan Yean (100%), 5′, Digby 1 (100%), S.A. (50%) and Major Mitchell Plateau (100%); and, 7′, Powlett River (40%), Tasmania (80%) and Drik Drik (100%). The trees from Powlett River and Cape Schanck were the most variable. The former were scattered through subgroups 2′, 5′, 6′ and 7′, and the latter into subgroups 3′ and 7′. *E. pauciflora* in subgroups 5′ and 6′ were characterized by having markedly small fruit (6.9 and 6.4 mm width respectively). Subgroup 5′ consisted of trees principally from Major Mitchell Plateau and Digby 1; while 6′ almost exclusively consisted of individuals from Wartook Reservoir, Grampians. It is interesting to note that, although fruit size of individuals from 5′ and 6′ are intermediate, their leaves are 0.35 mm thick, similar to those of *E. pauciflora* (0.35-0.43 mm) and different from peppermints which had relatively thin leaves (0.18-0.25 mm). Three trees from Mt. Buffalo were the only individuals in subgroup 4′, having broader, shorter leaves than the others (form factor 0.43). Subgroups 1′, 2′, 3′ and 7′ show similar measures over most leaf and fruit characters, although individuals in 2′ were characterized by glaucous stems and individuals in 7′ had sunken discs similar to *E. obliqua*.

Two ordinations are included, one ordinating individual trees and one the ten groups from the classification. The ordination of all individuals (Fig. 5) accounted for 41% of the total information in the first two axes and 51% on the first three. Individual trees of *E. radiata*, *E. willisii* and *E. obliqua* form recognizable clusters equivalent to the groups in the classification. These represent outgroups for interpreting the pattern within *E. pauciflora*. Fruit size was the most important character separating individuals on axis 1. Populations from Wartook Reservoir, Digby 1 and Major Mitchell Plateau are intermediate between the peppermints *E. radiata* and *E. willisii*, and the most distinctive largest fruited form of *E. pauciflora*. Individuals on axis two

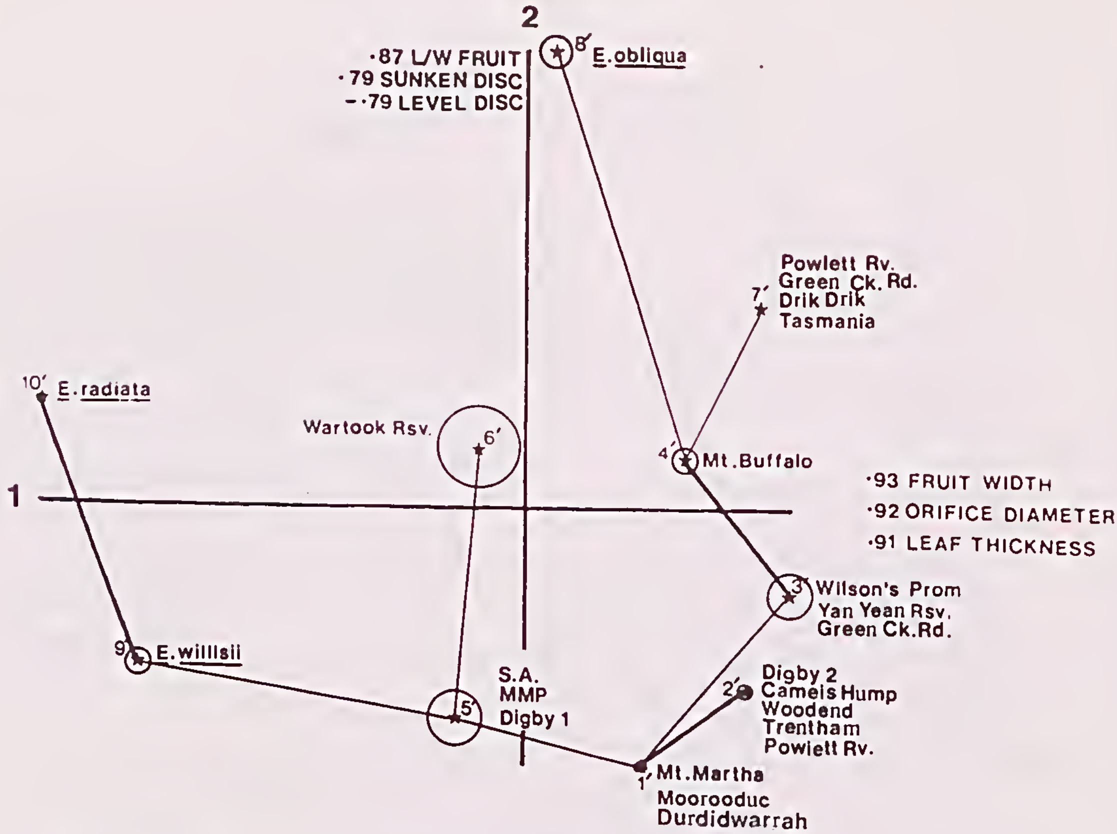

Fig. 6—Ordination of the group centroids of subgroups 1′-10′ defined by the classification of all adult individuals; MMP = Major Mitchell Plateau, S.A. = South Australia, L/W = length/width ratio. Links join nearest neighbours. Axis 3 is represented as the diameter of the spheres. Darker lines indicate a reciprocal link. Character correlation coefficients are shown on axes.

Fig. 7—Classification of seedlings of *E. pauciflora* (with some *E. obliqua* and *E. radiata*) truncated at the ten group level; N = node. General percentage composition of groups 1-10 is as follows. 1: Moorooduc 90%, Cape Schanck 50%, Mt. Martha 80%, Durdidwarrah 90%, Trentham 40%, South Australia 56%. 2: Digby 2 50%, Green Ck. Rd. 50%, Woodend 30%. 3: Powlett River 45%, Green Ck. Rd. 38%. 4: Cape Schanck 20%, *E. obliqua* 100%. 5: Woodend 50%, Mt. Buffalo 40%, Tasmania 40%, Camels Hump 40%, Trentham 20%. 6: Mt. Cole 86%, Major Mitchell Plateau 90%, Tasmania 60%, Mt. Buffalo 60%, Camels Hump 50%. 7: Wartook Reservoir 67%, *E. willisii* 78%. 8: *E. willisii* 22%, Wartook Reservoir 23%. 9: Powlett River 26%, Digby 1 75%. 10: *E. radiata* 90%, Powlett River 19%.

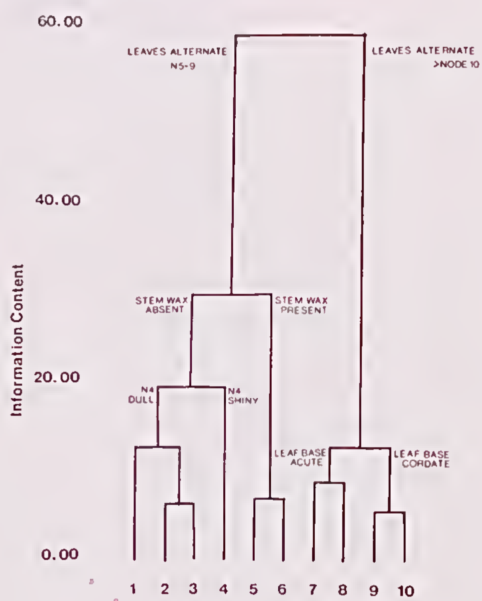

TABLE 4

CRAMER VALUES FOR CHARACTERS THAT BEST DISTINGUISH THE TEN MAJOR GROUPS DEFINED IN THE CLASSIFICATION OF THE SEEDLINGS. FOR QUALITATIVE CHARACTERS THE PERCENTAGE FREQUENCY OF SEEDLINGS IN EACH STATE IS GIVEN; FOR QUANTITATIVE CHARACTERS THE MEAN VALUE FOR THE CHARACTER IS GIVEN.

Groups	Characters			
	Node 4, dull/shiny		+ stem wax	
State	0	1	0	1
1	100%	0	100%	0
2	100	0	97.4	2.6
3	100	0	100	0
4	0	100	100	0
5	100	0	9.4	90.6
6	96.3	3.7	11.3	88.7
7	100	0	98.0	2.0
8	100	0	100	0
9	100	0	96.8	3.2
10	100	0	100	0
	Cramer value = 0.9388		Cramer value = 0.9075	

Groups	+ lignotuber		Node 7, petiole length (mm)
State	0	1	
1	0	100%	4.95
2	5.3	94.7	7.38
3	0	100	3.76
4	0	100	9.96
5	28	72	8.74
6	0	100	13.14
7	0	100	1.62
8	100	0	1.26
9	0	100	0.56
10	0	100	0.13
	Cramer value = 0.8495		Cramer value = 0.8488

Groups	Node 5, petiole length (mm)	Node 5, leaf width (mm)
1	2.23	47.14
2	2.81	56.92
3	0.88	53.14
4	3.97	43.27
5	3.14	49.25
6	5.64	58.47
7	0.34	33.20
8	0.68	36.05
9	0.08	39.23
10	0.00	31.12
	Cramer value = 0.7918	Cramer value = 0.7218

Groups	Node 5, form factor	Node 4, leaf width (mm)
1	0.82	35.62
2	0.75	48.08
3	0.80	41.14
4	0.65	38.38
5	0.79	40.87
6	0.79	49.36
7	0.59	33.22
8	0.65	32.41
9	0.75	34.33
10	0.62	27.44
	Cramer value = 0.7126	Cramer value = 0.7080

TABLE 4 *continued*

Groups	Characters			
	Node 4, petiole length (mm)	Node/leaves alternate		
	State	1(3-7)	2(8-9)	3(10-)
1	2.20	13.7%	74	12.3
2	1.83	68.4	29	2.6
3	0.79	29.7	56.7	13.6
4	2.40	93.3	6.7	0
5	2.45	62.5	37.5	0
6	3.27	75.5	24.5	0
7	0.33	0	4.0	96.0
8	0.77	4.5	13.6	91.9
9	0.50	0	16.1	83.9
10	0.00	0	5.5	94.5
	Cramer value = 0.7037	Cramer value = 0.6981		

separate on whether they have a level or sunken disc. As populations of *E. pauciflora* show variation in disc position they are separated on this axis. The ordination of the ten subgroups includes a minimum spanning tree linking nearest neightbours (Fig. 6). The first three axes account for 75% of the total variation. Fruit, leaf and bud characters are correlated with axis one. The interesting point of this ordination is the link between individuals from Wartook Reservoir and other small-fruited individuals in subgroup 5′. The intermediate position of the subgroups 5′ and 6′, and hence individuals from Wartook Reservoir, Major Mitchell Plateau and Digby 1, is also quite clear on the ordination. A reciprocal link between subgroups 1′ (Moorooduc, Mt. Martha and Durdidwarrah) and 2′ (Camels Hump, Woodend and Trentham), indicates similar morphology in these populations (fruits 8-9 mm long, 9 mm wide, leaves 0.37 mm thick). Trees within each of these groups are broadly speaking from the same geographic regions, and so may be subject to similar climatic and edaphic conditions.

SEEDLINGS

The classification of all seedlings (Fig. 7) was truncated at the ten group level. The major dichotomy was based on the node at which leaves alternate. Groups 1-6 consist of ash-like seedlings (alternation nodes 5-10), and groups 7-10 of peppermint-like seedlings (alternation nodes 8-indefinite). Within the ashes, all seedlings from localities of 1,000 m altitude and above, from Tasmania, and a few from Woodend occur in groups 5 and 6. As GCOM and CRAMER indicate (Table 4), these populations are characterized by all seedlings being glaucous. Groups 1-3 consist virtually of all lowland populations of *E. pauciflora* (percentage frequency of population composition for these three groups is shown in the caption to Fig. 7).

Three ordinations of the seedling data were subsequently run. The ordination of the ten groups generated by the classification showed many characters highly positively correlated with axis one, although groups were principally separated on petiole length and the node at which intranode formation occurs (Fig. 8). An important point illustrated in this ordination is the presence of seedlings from Powlett River in groups 3 (*E. pauciflora*), 9 (Digby 1—intermediate) and 10 (*E. radiata*). Seedlings from individuals were split between these three groups, indicating character segregation, and hence hybridisation at this locality. Cape Schanck seedlings occurred in groups 3 and 4 (the latter including *E. obliqua*), again strongly indicating recent hybridisation. Wartook Reservoir seedlings which had questionable parentage were very similar to peppermint seedlings in having opposite, blue-green leaves for many nodes. They showed, however, distinct ash characters of petiole formation and a change in leaf orientation around nodes 7-8 (Fig. 9). There was, however, no sign of segregation, suggesting recent hybridisation is less likely. The position of groups 5 and 6 (higher altitude) on the plot indicate they generally have longer petioles and alternate at an earlier node than the lower altitude seedlings. The ordination of seedlings from the fourteen populations (Fig. 10), excluding those showing recent hybridisation, further highlighted the separation between lower and higher altitude sites. The program NEAREST indicated a very weak link between groups 4 (Trentham) and 14 (Camels Hump), as shown by the dashed line. This program separates all populations from above approximately 1,000 m and Tasmania from all lowland populations. Again, high altitude seedlings show longer petioles (10-15 mm at node 7) and earlier alternation (generally nodes 5-7) than the lower altitude seedlings which show petioles 2-8 mm in length at node 7 and alternation ranging from nodes 5-10 (most showing later alternation around nodes 8-10; see Fig. 11). Of the total variation in the ordination of Fig. 10, 72% was retained in the first three axes. One of the most important characters separating populations is the presence of wax, all high altitude individuals, apart from a few from Major Mitchell Plateau, being glaucous. Regional variation seen in the adults is reflected in the seedlings, with some clustering around the Mornington Peninsula/Durdidwarrah and Woodend/Camels Hump districts. Looking at characters over all populations, petiole length at node 7

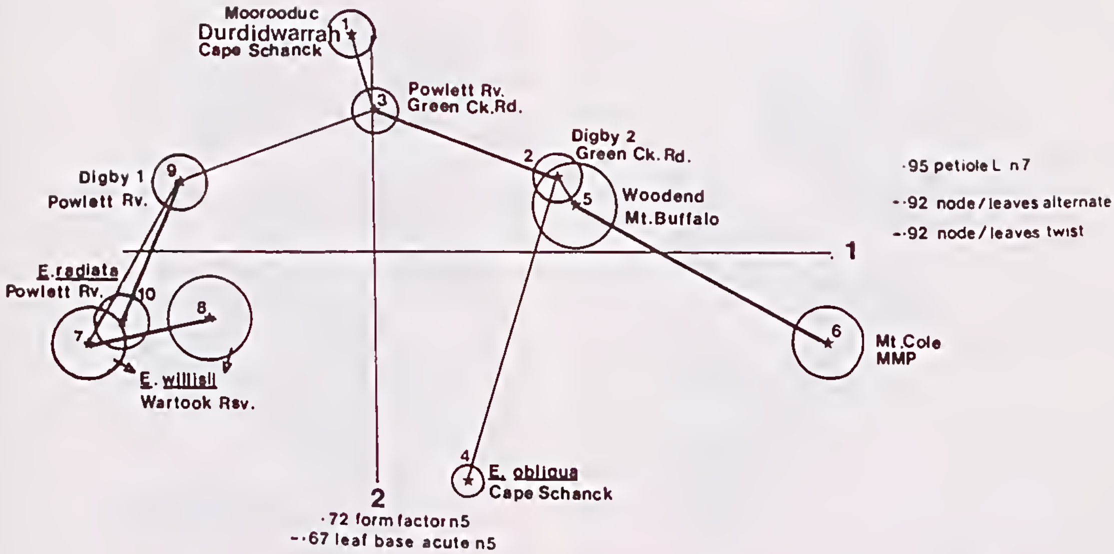

Fig. 8—Ordination of group centroids of the ten groups defined in the classification of all seedlings. Links join nearest neighbours; axis 3 is represented as the diameter of the spheres; with character correlation shown on axes 1 and 2. For complete group composition see caption of Fig. 7; MMP = Major Mitchell Plateau, L = length, N = node.

Fig. 9—Seedlings from individuals of: (A) *E. willisii*, Mirranatwa Gap, Grampians and (B) the equivocal population at Wartook Reservoir, Grampians. Scale: 5 cm.

varied quite markedly, ranging from 2 mm in South Australia to 15 mm at Mt. Buffalo. Apart from the South Australian population, western district populations separate on axis two, most possessing raised oil glands on the margin of node 4. The significance of this character is uncertain. The displacement of the South Australian population on axis three, on which oil gland density is negatively correlated (Table 5), is due, in part, to a low oil gland density (23 cm^{-2}).

Populations from the Grampian Ranges warranted special attention, consequently all individuals from that region were ordinated separately, with 49% of the total variation being accounted for in the first three axes (Fig. 12). Seedlings of *E. pauciflora* collected at two localities are relatively distinct, again showing a trend to longer petioles and earlier intranode formation at higher altitudes, as shown by the correlation coefficients (Fig. 12). There is, however, some overlap, indicating a morphological gradient between the two forms. Open circles in Fig. 12 indicate seedlings from individuals thought to be *E. willisii*, collected at Mirranatwa Gap; the seedlings from Wartook Reservoir (closed circles) are quite peppermint-like, emphasised by the overlap of the open and closed circles. Compared to the spread in seedlings of *E. pauciflora* on this plot, the Wartook Reservoir individuals show consistent characters. Leaf length is positively correlated with axis three (Table 8), the seedlings of *E. pauciflora* generally having longer leaves (113-125 mm, node 5) than the Wartook Reservoir individuals (95.7 mm, node 5).

DISCUSSION

Regional Patterns

Lowland populations of *E. pauciflora* were relatively similar overall, most being of woodland form and gum-barked to the base, although trees at Trentham were of forest form and some others had a rough-barked butt. Fruits ranged between 8-11 mm wide and disc position varied within populations from level to sunken. Leaf length varied (114 mm at Camels Hump to 175 mm at Digby), but overall leaves were narrow (average of 25 mm), slightly falcate, acuminate with a hook, and showing distinctive parallel veination. Buds (7-15 per axillary cluster) remained relatively consistent in shape throughout the range). All populations from higher altitudes (e.g. Camels Hump), had glaucous fruits and stems as well as some trees from Digby (120-160 m a.s.l.). The relative similarity of individuals over all populations at lower altitudes suggests a general uniformity of habitat and therefore, lack of intense selection pressures at lower altitudes. At higher altitudes populations of *E. pauciflora* show variation in a number of physiological and morphological characters with changing elevation. Thus the species seems to have the capacity to adapt in response to different habitats. Pryor (1957) suggested that when looking at the association between growth rate and altitude of origin, other factors beside elevation come into effect at lower altitudes. This is probably also the case with other morphological and physiological parameters.

The similarity of some populations of *E. pauciflora*, identified as regional forms, may be due to similar environmental conditions. Durdidwarrah and Moorooduc populations both occur on Tertiary acid, sandy-clay sediments (Douglas & Ferguson 1976) providing similar growth substrates. These populations have fruits about 8 mm long, thick leaves and are non-glaucous. It is this last character that appears to differentiate the Mornington Peninsula populations from those in the Wood-

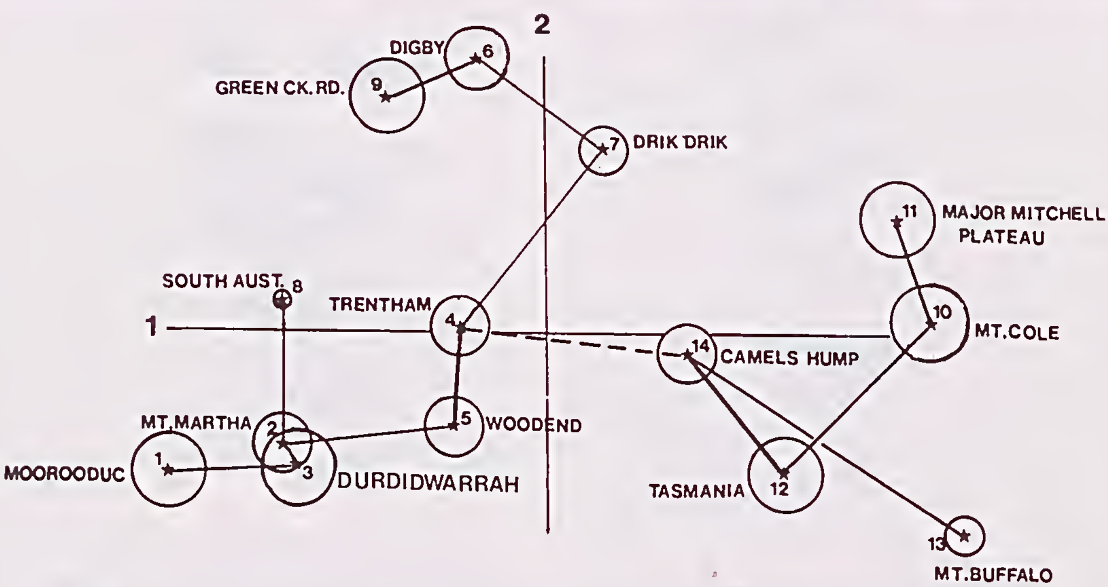

Fig. 10—Ordination of the fourteen seedling populations. Links join nearest neighbours. The dashed line indicates a weak link. Axis 3 is represented as the diameter of the spheres.

Fig. 11—Seedlings of *E. pauciflora* grown from individuals collected at: (A) Digby, (B) Mt. Martha, (C) Major Mitchell Plateau, (D) Mt. Buffalo, illustrating the differences in intranode formation and petiole length at the different altitudes. Scale: 5 cm.

TABLE 5
THE MOST IMPORTANT CHARACTERS (FROM BACRIV) THAT CORRELATE WITH AXES ONE, TWO AND THREE OF THE ORDINATION OF THE FOURTEEN SEEDLING POPULATIONS OF *E. pauciflora*

	Character	Correlation coefficient
Vector 1	Node 7, petiole length (mm)	0.9384
	Node 5, petiole length (mm)	0.9240
	Node 4, blue green	0.8872
	Node/leaves twist	−0.8095
	Stem wax present	0.8047
Vector 2	Node 4, raised oil glands on leaf margin	0.8009
	Node 4, leaf length (mm)	0.7182
	Node 6, blue green	−0.7087
Vector 3	Oil gland density (cm^2)	−0.5636

end district. Apart from the glaucous character, populations from the two areas show similar adult morphology, indicating perhaps a widespread regional form. The reflection of this regional variation in the seedlings suggests, in part, a genetic basis for the pattern. Pryor (1976) noted the diagnostic importance of juvenile leaves, stating that they were useful markers in genetic analysis. Consequently the variation in seedling characters is an important factor when looking at the overall pattern. The differentiation between low and higher altitude seedlings was seen in each analysis. With minor exceptions, seedlings from populations from 1,000 m or above, or those from Tasmania with a colder climate, were glaucous and generally showed a more rapid transition to intermediate foliage (nodes 5-7) and longer petioles (10-14.6 mm) than did lower altitude seedlings (nodes 6-10, 3.8-8.0 mm) (Fig. 11). Other minor variations included leaf shape, such as some seedlings from Moorooduc having more orbicular leaves than those from higher altitudes. Trees from Major Mitchell Plateau (Grampians) produced typical higher altitude seedlings of *E. pauciflora*, with long petioles, rapid transition to intermediate foliage and glaucousness. Considering seedlings are such useful genetic markers, it appears that the small fruits of trees from the Major Mitchell Plateau are due to variation within *E. pauciflora* rather than hybridisation with *E. willisii*, as there appears to be no other peppermint characters evident.

Using information from a cladistic analysis of *Monocalyptus* (Ladiges, Humphries & Brooker 1983), outgroup comparison suggests that earlier transition to intermediate foliage is a derived (advanced) character. Thus lower altitude seedlings are perhaps showing the ancestral condition. This is interesting in relation to previous suggestions of a single gene pool (Slatyer & Morrow 1977), and a widespread lowland population of *E. pauciflora* during the last glaciation (Dodson 1975). It is not possible, of course, to say unequivocally what the evolutionary history has been, but one alternative is that, with the advent of the warmer Holocene, some of the lowland populations of *E. pauciflora* migrated and adapted to higher altitudes. The present-day, isolated, lowland populations are possibly relics, their demise being due, in part, to competition from other, more vigorous, eucalypts adapted to the warmer conditions.

RECENT HYBRIDISATION

Variable adult characters in individuals from Powlett River and Cape Schanck suggested recent hybridisation. Seedling segregation strongly indicated hybridisation was occurring between *E. pauciflora* and *E. obliqua* at Cape Schanck, and between *E. pauciflora* and *E. radiata* at Powlett River. Trees of the putative parental species were present at each site.

INTROGRESSION?

The equivocal population from Wartook Reservoir, Grampians, is not as readily explained as other morphologically different populations. Adults had small, peppermint-like fruits 6-7 mm long yet leaves showed parallel veination, a distinct Snow Gum character. Leaf thickness approached that of *E. pauciflora* in comparison to the relatively thin peppermint leaves. Bark was variable, ranging from totally gum-barked to decorticating bark on the primary branches. Data from the seedling trial proved even more interesting. Juvenile leaves were narrow, blue-green, and opposite for many nodes and in this respect were very similar to seedlings grown from the peppermint, *E. willisii* (from individuals collected elsewhere in the Grampians). The Wartook Reservoir seedlings, however, became petiolate around node 8, sometimes earlier, and showed orientation in the vertical plane, also around node 8 (both ash characters). Furthermore, seedling characters were relatively consistent, with no suggestion of segregation. The possession of some distinctive ash-like characters in what overall appears to be a peppermint, raises the question of whether introgressive hybridisation has played a part in the evolution of the Wartook Reservoir population. The extent of the gene transfer in Grampians populations, the geographic range and the pattern of variation of this form need close study before the evolutionary and taxonomic implications can be resolved. It is noted in passing that *E. vitrea* R. T. Baker, described from a

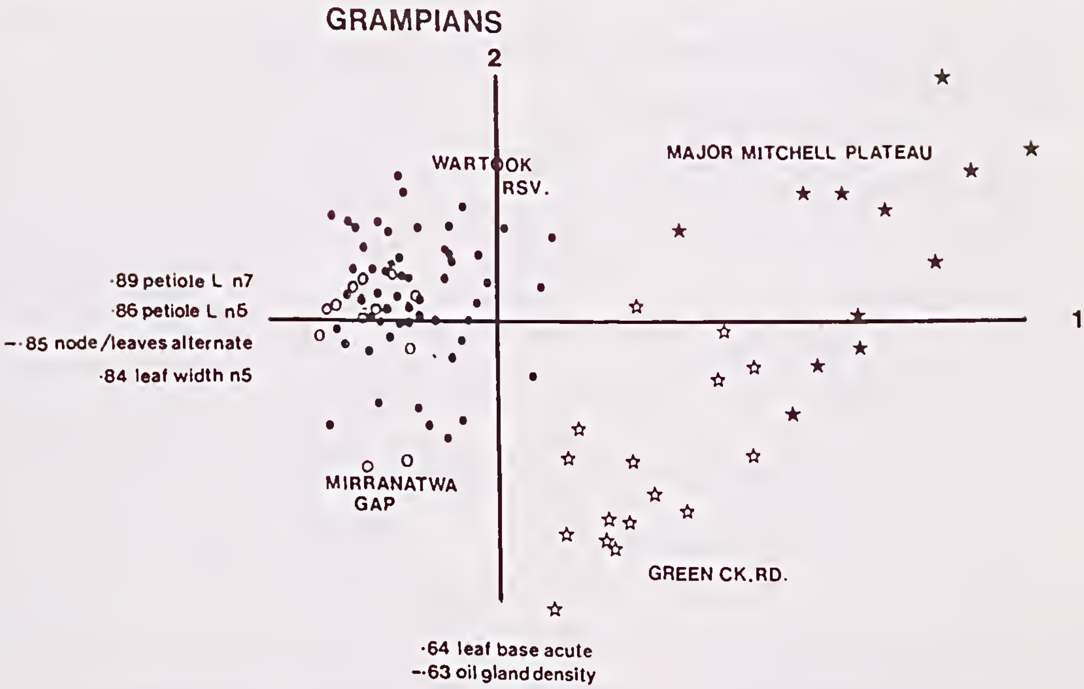

Fig. 12—Ordination of all seedlings from populations in the Grampians; character correlation coefficients are shown on axis 1 and 2; L = length, N = node. Stars indicate *E. pauciflora* seedlings, open circles seedlings from *E. willisii* at Mirranatwa Gap, and closed circles, the equivocal form collected at Wartook Reservoir.

population in Crookwell, N.S.W. (Baker 1900), has been applied to some populations in the Grampians (Sibley 1967, Parsons, Scarlett & Rosengren 1972). However, the type specimen has been described as a putative hybrid between *E. pauciflora* and *E. radiata* (Pryor & Johnson 1971). If specific rank were appropriate for the Wartook Reservoir population, then the epithet *vitrea* should probably not be used.

CONCLUSION

Overall similarity in adult morphology of lowland populations suggests less intense selection pressures are acting at lower elevations. Evolutionary implications are discussed, taking into consideration the scattered nature of the populations (possibly relics) and the differences in seedling morphology between 'low' and 'high' altitude forms. *E. pauciflora* probably was quite widespread at lower altitudes during the last glaciation. At some stage *E. pauciflora* may have been involved in the evolution, by introgression with *E. willisii*, of a stable form at Wartook Reservoir (Grampians), but extensive sampling is required for corroboration.

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APPENDIX 1

LOCALITIES OF POPULATIONS OF *E. pauciflora*; INCLUDING INDIVIDUAL TREE NUMBERS.

Site	Rainfall mm	Soil/geology	Altitude M	Associated eucalypts
1-5 Moorooduc, Frankston-Flinders Rd., Calder's (1972) site No. 34.	762	Yellow podzol/ Tertiary sandstone	46	*E. ovata*
6-10 Cape Schanck, Calder's (1972) site No. 186.	762	Yellow podzol	76	*E. obliqua*
11-15 Mt. Martha, Forest Dve., Calder's (1972) site No. 65.	762	Yellow podzol/ granite	152	*E. ovata* *E. viminalis*
16-20 Durdidwarrah, on road to Meredith.	550	Laterite capping/ Tertiary sediments	380	–
21-25 Trentham, 2.2 km west to Tylden, Trentham Rd.	762-1016	Basalt	640	*E. viminalis*
26-30 Woodend, 4-5 km east of Carlsruhe, on Newham Rd.	762	Sandstone/ mudstone	540	*E. rubida*
31-40 Powlett River, "Manuka Ridge" property of Clive Hollins.	762-1016	Old sand-dune system/coffee rock	0-20	*E. viminalis* *E. ovata,* *E. radiata*
41-43 Digby 1, 1.5 km along the Digby-Strathdownie Rd.	762-1016	Sandstone	140-160	*E. ovata* (1 & 2) *E. baxteri* (1)
44-47 Digby 2, 4 km along the Digby-Strathdownie Rd.	762-1016	Sandstone	140-160	*E. ovata* (1 & 2) *E. baxteri* (1)
48-50 Drik Drik, 4.8 km S.E. of Drik Drik.	762-1016	Inland dunes	140-160	*E. ovata*
51-54 South Australia Caroline State Forest, south of "Broomfield" off Caroline Rd.	762-1016	Inland dunes	140-160	*E. baxteri* *E. viminalis*
55-64 Wartook Reservoir Grampians. Bounded by Smith Rd. and Roses Creek Rd.	760-780	Sandstone	440-480	*E. viminalis* *E. baxteri* *E. obliqua*
65-69 Green Ck. Rd. 3.8 km N., junction of Victoria Valley Rd. and Green Ck. Rd., Grampians.	510	Granite	260	*E. aromaphloia* *E. obliqua*
70-73 Mt. Cole, Western Victoria.	762	Granite	914	–
74-78 Major Mitchell Plateau.	760-780	Sandstone	1017	*E. alpina*
79-83 Tasmania, Eildon near Hungry Flats.	762	Triassic stratified rock	470	–
84-88 Mt. Buffalo National Park, near carpark and chalet.	>1500	Granite	1470	–
89-92 Camels Hump, near Mt. Macedon.	762	Volcanic	1011	–

APPENDIX 1 *continued*

Site	Rainfall mm	Soil/geology	Altitude M	Associated eucalypts
93-97 Wilsons Promontory north of the National Park, near Vereker Ranges.	760-780	Old sand-dunes	10-20	*E. viminalis* *E. obliqua*
98-102 Yan Yean Reservoir catchment area, track 3.	762-1016	Alluvium	200	*E. ovata*

* Rainfall data taken from Watt (1937).